

HY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Neil Fraser Fairweather et al.
 Title: TETANUS TOXIN POLYPEPTIDES
 Appl. No.: 10/018,997
 Filing Date: December 26, 2001
 Examiner: Unassigned
 Art Unit: Unassigned

SUBSEQUENT PRELIMINARY AMENDMENT

Commissioner for Patents
 Box PCT
 Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application, Applicants respectfully request that the above-identified application be amended as follows:

In the Specification:

Please amend the Specification as shown:

Please delete the paragraph at page 21, lines 7-19 and replace it with the following paragraph:

E. coli strain BL21 (λ DE3, *ompT*, *hsdS_B* (*r_B-m_B-*), *gal*, *dcm*) was used as the host for the plasmids described below. Plasmid pKS1 contains a codon-optimised gene for the expression of the Hc fragment of TeNT under the control of the T7 promoter. It was created by PCR amplification (Pfu polymerase, Stratagene, Cambridge UK) of a 1357 bp fragment using pTETtac215 (Makoff *et al.*, 1989) as template and the oligonucleotides 5'GAGCATATGAAAAACCTTGAT (SEQ ID NO: 2) and 5'CGGATCCTTAGTCGTTGGTCCA (SEQ ID NO: 3) which introduce *Nde*I and *Bam*HI

sites at the 5' and 3' ends of the gene respectively. After blunt end ligation of the PCR product into the vector pCRScript (Stratagene) to form plasmid pJC6, the *NdeI* – *Bam*HI fragment was purified by agarose gel electrophoresis using a Qiaex II gel purification kit (Qiagen, West Sussex, UK), and subcloned into pET28a (Novagen, Cambridge UK) which has previously been digested with *NdeI* and *Bam*HI (Roche Molecular Biochemicals, East Sussex, UK). DNA manipulations were performed by standard procedures.

Please delete the TABLE 1 Mutants of TeNT Hc constructed at page 22, and replace it with the following TABLE:

TABLE 1 Mutants of TeNT Hc constructed

Mutant Name	Oligonucleotides used for site directed mutagenesis
M5	NF38: 5' to 3' GGTGCGACTGGTACTTCTAAGGATCCGAATTCG (SEQ ID. NO:4) NF41: 3' to 5' CGAATTCGGATCCTTAGAAGTACCAAGTCGCAACC (SEQ ID. NO:5)
T1308A	NP49: 5' to 3' GACTGGTACTTCGTTCCGGCTGATGAAGGTTGGA (SEQ ID. NO:6) NP50: 3' to 5' GGTCCAACCTTCATCAGCCGGAACGAAGTACCAG (SEQ ID. NO:7)
D1309A	NF51: 5' to 3' TGGTACTTCGTTCCGACCGCTGAAGGTTGGACGA (SEQ ID. NO:8) NF52: 3' to 5' CGTTGGTCCAACCTTCAGCGGTCGGAACGAAGTA (SEQ ID. NO:9)
E1310A	NF57: 5' to 3' TACTTCGTTCCGACCQATGCTGGTTGGACGCAACGAC (SEQ ID. NO:10) NF58: 3' to 5' GTCGTTGGTCCAACCAAGCATCGGTCGGAACGAAGTA (SEQ ID. NO:11)
M13	NF47: 5' to 3' TTCGTTCCGACCGATGAATAAGGATCCGAATTCG (SEQ ID. NO:12) NF48: 3' to 5' CGAATTCGGATCCTTATTCATCGGTCGGAACGAA (SEQ ID. NO:13)
M28	NF79: 5' to 3' GGTACCCACAACGTGTGACGCAACCGTGACATCCTG (SEQ ID. NO:14) NF80: 5' to 3' CAGGATGTCACGGTTCGGCTGACCGTTGTGGGTACC (SEQ ID. NO:15)
M37	NF81: 5' to 3' CTGGGTCTGGTTGGTACCAACGACCCGAACCGTGAC (SEQ ID. NO:16) NF82: 5' to 3' GTCACGGTTCGGGTCGTTGCTACCAACCAGACCGAG (SEQ ID. NO:17)
M40	NF79: 5' to 3' GGTACCCACAAGGTCAGCCGAACCGTGACATCCTG (SEQ ID. NO:18) NF80: 5' to 3' CAGGATGTCACGGTTCGGCTGACCGTTGTGGGTACC (SEQ ID. NO:19) and NF32: 5' to 3' CTTCTAACTGGTACTTCAACTCTCTGAAAGACAAAATCCTGGG (SEQ ID. NO:20) NF33: 3' to 5' CCCAGGATTTTGTCTTTCAGAGCGTTGAAGTACCAGTTAGAAG (SEQ ID. NO:21)
M58	NF91: 5' to 3' GTTGGTTACCCGAAACTGCAGAACCTGGACAGAATT (SEQ ID. NO:22) NF92: 3' to 5' AATTCTGTCCAGGTTCTGCAGTTTCGGGTAACAAC (SEQ ID. NO:23)
M564	NF32: 5' to 3' CTTCTAACTGGTACTTCAACTCTCTGAAAGACAAAATCCTGGG (SEQ ID. NO:24) NF33: 3' to 5' CCCAGGATTTTGTCTTTCAGAGCGTTGAAGTACCAGTTAGAAG (SEQ ID. NO:25)
M567	NF97: 5' to 3' CTAAGTGGTACTTCAACGCTCTGAAAGAGAAAATCCTGGG (SEQ ID. NO:26) NF98: 3' to 5' CCCAGGATTTTGTCTTTCAGAGCGTTGAAGTACCAGTTAG (SEQ ID. NO:27)

Please delete the Amino acid sequence of *C. tetani* neurotoxin (TeNT)
constructed at page 36, and replace it with the following TABLE:

Amino acid sequence of *C. tetani* neurotoxin (TeNT)

1	mpitinnfry	sdpvnndtii	mmeppyckgl	diyykafkit	driwivpery	efgtkpedfn
61	ppssliegas	eyydpnylrt	dsdkdrflqt	mvklfnrikn	nvagealldk	iinaipylgn
121	syslldkfdt	nsnsvsfnll	eqdpsgattk	samltnliif	gpgpvlnkne	vrgivlrvidn
181	knyfpcrdgf	gsimqmafcg	eyvptfdnvi	enitsltigk	skyfqdpall	lmheliwhlh
241	glygmqvssh	eiipskqeyi	mghtypisae	elftfggqda	nlisidiknd	lyektlndyk
301	aiannklsqvt	scndpnidid	sykqiyyqky	qfdkdsnggy	ivnedkfqil	ynsimygfte
361	ielgkkfnik	trlsyfsmnh	dpvkipnld	dtiyndtegf	nieskdlkse	ykgqnmrvnt
421	nafrnvdgsg	lvskliglck	kiipptnire	nlynrtaslt	dlggelciki	knedltfaie
481	knsfseepfq	deivsyntkn	kplnfynsl	kiivdynlqs	kitlpndrtt	pvtkgipyap
541	eyksnaasti	eibhiddnti	yqylyaqksp	ttlqritmtn	svddalinst	kiysyfpsvi
601	skvnqgaqgi	lflqwvrdii	ddftnessqk	ttidkisdvs	tivpyigpal	nivkqgyegn
661	figalettg	vllleyipei	tlpviaalsi	aesstqkeki	iktidnflek	ryekwievyk
721	lvkakwlg	ntqfqkrsg	myrsleyqvd	aikkiidyey	kiysgpdkeq	iadeinnlkn
781	kleekankam	ininifmres	srsflvngmi	neakkqllef	dtqsknilmq	yikanskfig
841	itelkkiesk	inkvfstpip	fsysknldcw	vdneedidvi	lkkstilnid	inndiisdis
901	gfnssvityp	daqlvpging	kaihlvnnes	sevivhkamd	ieyndmfnnf	tsvfwlrvpk
961	vsashlegyg	tneysiissm	kkhsisigsg	wsvslkgnnl	iwtlkdsage	vrqitfrdlp
1021	dkfnaylank	wvfititndr	lssanlying	vlmgsaeitg	lgairednni	tlklrdcnnn
1081	nqyvsidkfr	ifckalnpe	ieklytsyls	itflrdfwgn	plydteyyyl	ipvassskdv
1141	qlkahtdytny	ltnapsytng	klniyyrrly	nglkfiikry	tpnneidsfv	ksgdfiklyv
1201	synnnehivg	ypkdgnafnn	ldxilrvgyn	apgiplkykm	eavklrdikt	ysvqlklydd
1261	knasglvg	hngqigndpn	rdiliasnwy	fnhlkdkiig	cdwyfvptde	gwtnd

(SEQ ID NO: 1)